American Journal of Plant Sciences, 2017, 8, 441-450
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Enzymatic Synthesis and Structural Characterization of Rebaudioside D3, a Minor Steviol Glycoside of *Stevia rebaudiana* Bertoni

Guohong Mao[1*], Venkata Sai Prakash Chaturvedula[2], Jacob Edward Vick[1], Oliver Yu[1,3]

[1]Conagen Inc., Bedford, MA, USA
[2]Blue California, Rancho Santa Margarita, CA, USA
[3]Wuxi New Way Biotechnology Ltd., Wuxi, China
Email: *guohong.mao@conagen-inc.com

How to cite this paper: Mao, G.H., Chaturvedula, V.S.P., Vick, J.E. and Yu, O. (2017) Enzymatic Synthesis and Structural Characterization of Rebaudioside D3, a Minor Steviol Glycoside of *Stevia rebaudiana* Bertoni. *American Journal of Plant Sciences*, **8**, 441-450.
https://doi.org/10.4236/ajps.2017.83030

Received: December 29, 2016
Accepted: February 6, 2017
Published: February 9, 2017

Abstract

Rebaudioside D3, a novel steviol glycoside, is produced by specific UDP-glycosyltransferase of rebaudioside E, a minor steviol glycoside of *Stevia rebaudiana* Bertoni. The complete proton and carbon NMR spectral assignments of rebaudioside D3, 13-[(2-*O*-*β*-D-glucopyranosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) ester, was achieved by the extensive 1D and 2D NMR (^{1}H and ^{13}C, TOCSY, HMQC, HMBC) as well as mass spectral data. Further, hydrolysis studies were performed on rebaudioside D3 using acid and enzymatic studies to identify aglycone and sugar residues in its structure. Rebaudioside D3 is detected in the commercial extract of the leaves of *Stevia rebaudiana* by LC-MS analysis, suggesting rebaudioside D3 is a natural steviol glycoside.

Keywords

Stevia rebaudiana, Diterpene Glycoside, Structure Characterization, Spectral Data, LC-MS

1. Introduction

Stevia rebaudian Bertoni (Bertoni), a perennial shrub of Asteraceae (Compositae) family member native to South America, has been historically utilized to produce sweet beverages. The sweetness of *S. rebaudiana* is due to the presence of various diterpene glycosides namely steviolbioside, stevioside, rebaudiosides A-E, dulcoside A, and rubusoside, which are glycosides of the diterpene, steviol (*ent*-13-hydroxykaur-16-en-19-oic acid) [1]-[6]. Due to the increasing demand in the utilization of extracts from *S. rebaudiana* and its glycosides, it is now grown com-

mercially in Japan, Singapore, Malaysia, South Korea, China, and India [7] [8].

Recent studies reported identification and structural characterization of several minor diterpene glycoside compounds from various commercial stevia extracts [9] [10]. In this paper, we are reporting a new compound, rebaudioside D3, obtained by the enzymatic conversion of rebaudioside E. The same compound was identified from the crude extract of *Stevia rebaudiana* using comparative HPLC and LC-MS.

The majority of steviol glycosides are formed by a series of glycosylation reactions of steviol, the backbone molecule of the majority of isolated steviol glycosides. These glycosylation are typically catalyzed by the UDP-glycosyltransferases (UGTs) using uridine 5'-diphosphoglucose (UDP-glucose) as a donor of the sugar moiety [11] [12]. UGTs in plants make up a very diverse group of enzymes that transfer a glucose residue from UDP-glucose to steviol. For example, glycosylation of the C-3' of the C-13-O-glucose of stevioside yields rebaudioside A; and glycosylation of the C-2' of the C-19-O-glucose of the stevioside yields rebaudioside E [13].

In order to discover natural steviol glycoside sweeteners, we screened UGTs by enzymatic assay and identified a specific UGT that performs glycosylation specifically on the C-6' of the C-13-O-glucose. Thus, rebaudioside E is treated with this UGT to produce a novel steviol glycoside, named here as rebaudioside D3 (Reb D3 (1), **Figure 1**). We are herewith describing the production, isolation, characterization, and complete ^{1}H and ^{13}C NMR spectral assignments for the diterpene glycoside of Reb D3 which was assigned as 13-[(2-*O*-*β*-D-glucopyra-

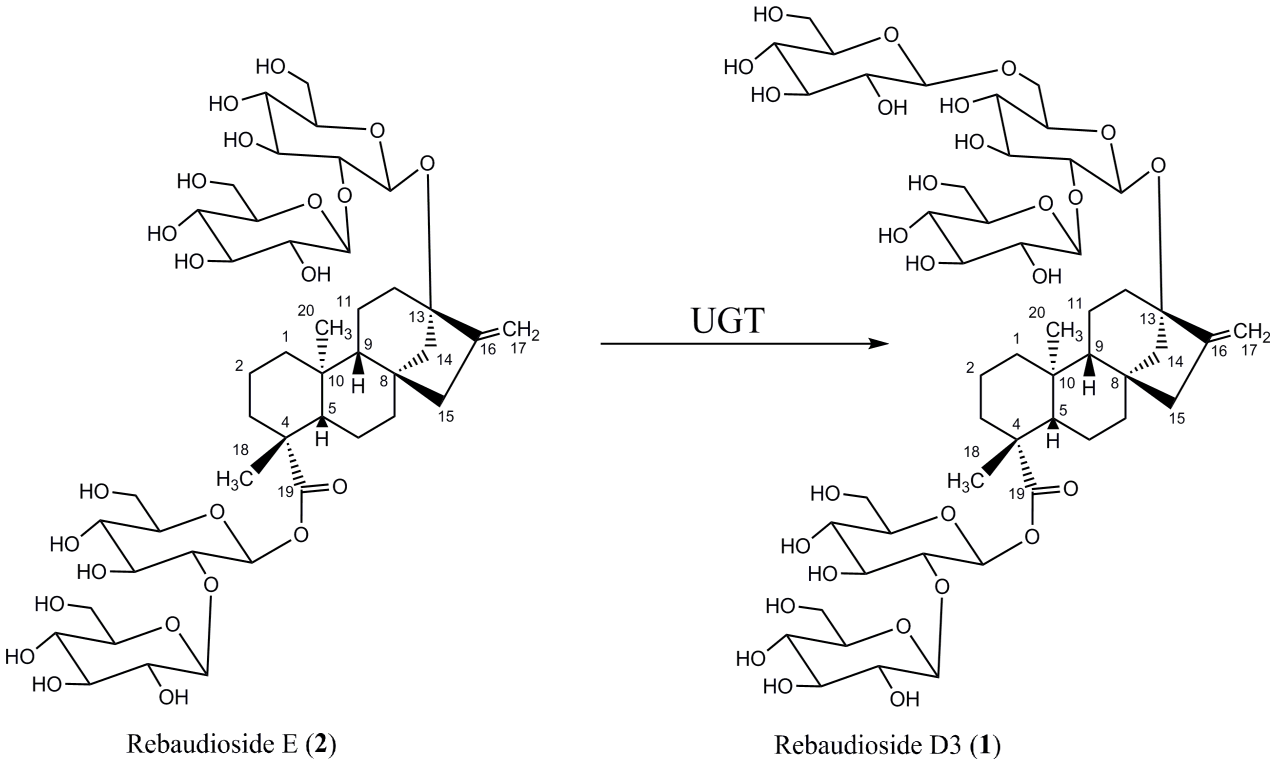

Figure 1. Bioconversion of rebaudioside E (**2**) to rebaudioside D3 (**1**) using glycosyltransferase.

nosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) ester] (**1**). The complete NMR assignments were achieved on the basis of 1D (^{1}H and ^{13}C) and 2D (TCOSY, HMQC and HMBC) NMR as well as high resolution mass spectroscopic data. Acid and enzymatic hydrolysis studies on **1** were carried out to identify aglycone and sugar residues. Further, the same compound was also detected from the leaf extract of *S. rebaudiana* by LC-MS and was confirmed by the comparative retention time of their HPLC profiles, indicating the natural existence of rebaudioside D3.

2. Experimental

2.1. Materials

The material used for the characterization of rebaudioside D3 was produced by enzymatic conversion of rebaudioside E. The commercial extract of *S. rebaudiana* leaves were supplied by Blue California (Rancho Santa Margarita, CA) and the authenticity of the commercial extract was confirmed by performing its retention time (t_R) comparison with the internal standard compounds of known JECFA steviol glycosides using the preparative HPLC method as reported earlier [4] [5] [6]. A voucher specimen of stevia leaf extract is deposited at Blue California. The main composition in the extract is stevioside, rubaudioside A, E and other steviol glycosides.

2.2. Enzymatic Synthesis and Isolation of Rebaudioside D3

Rebaudioside D3 was isolated from bioconversion of rebaudioside E by a proprietary glucosyltransferase from Conagen Inc. The bioconversion reaction mixture contains 1 mg/mL rebaudioside E, 3 mM $MgCl_2$, 1 mM UDP-glucose, 50 μg/ml UDP glycosyltransferase and 50 mM potassium phosphate buffer (pH 7.2). The reaction was performed at 30˚C and terminated by adding *n*-butanol after 24 hr. The samples were extracted with *n*-butanol by vortexing for 20 min at room temperature, and the butanol fraction was concentrated, dried and dissolved in 80% methanol for further purification. Compound **1** was purified by repeated isocratic elution (70% acetonitrile in water) of the above crude mixture dissolved in aqueous methanol using a Dionex UPLC ultimate 3000 system with a Phenomenex Luna NH_2 column (250 × 4.6 mm, 5 μm, 100 Å) by collecting the peak at the retention time (t_R) 6.77 min. Subsequent drying of the resultant solution under nitrogen yielded compound **1**.

2.3. General Instrumentation Methods

HPLC analysis was performed using a Dionex UPLC Ultimate 3000 system (Sunnyvale, CA), equipped with a quaternary pump, a temperature controlled column compartment, an auto sampler, and a UV absorbance detector. Phenomenex Luna NH_2 with guard column, 150 × 3.0 mm, 3 μm (100 Å) or Synergi Hydro-RP column (100 × 3.0 mm, 2.5 μm (100 Å)) were used for the characterization of rebaudioside D3 (**1**) by monitoring 210 nm. NMR spectra were ac-

quired on a Varian INOVA 600 MHz instrument with a 5 mm HCN probe using standard pulse sequences. The NMR spectra were performed in pyridine-d_5 (C_5D_5N); chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. The spectral data was referenced to the residual solvent signal (δ_H 7.19, and δ_C 123.5 for pyridine-d_5). IR spectral data was acquired using a Perkin Elmer 400 Fourier Transform Infrared (FT-IR) Spectrometer with Universal Attenuated Total Reflectance (UATR) polarization accessory. MS and MS/MS data were generated with a Thermo LTQ-FTMS mass spectrometer (100,000 resolution) equipped with a Nano spray ionization source. Samples were diluted with methanol and introduced via infusion using the onboard syringe pump.

2.4. Acid Hydrolysis of Rebaudioside D3

Rebaudioside D3 (5mg) was suspended in 10mL of MeOH and refluxed for 24 hours by the addition of 3 ml of 5% H_2SO_4. The reaction mixture was then neutralized with saturated sodium carbonate and extracted with ethyl acetate (EtOAc) (2 × 25 ml) to give an aqueous fraction containing sugars and an EtOAc fraction containing the aglycone component. The aqueous phase was concentrated and compared with standard sugars using the TLC systems EtOAc/ n-butanol/water (2:7:1) and CH_2Cl_2/MeOH/water (10:6:1) [14] [15] [16].

2.5. Enzymatic Hydrolysis of Rebaudioside D3

Compound **1** (1 mg) was dissolved in 10 ml of 0.1 M sodium acetate buffer, pH 4.5 and crude pectinase from *Aspergillus niger* (50 uL, Sigma-Aldrich, P2736) was added. The mixture was stirred at 50˚C for 96 hr. The product precipitated out during the reaction and was filtered and then crystallized. The resulting product obtained from the hydrolysis of **1** was identified as steviol by comparative TLC as well as ^{1}H NMR spectral data [17].

2.6. Liquid Chromatography and Mass Spectrometric Analysis of Stevia Extract

In order to detect the existence of rebaudisodie D3 in the commercial extract of the leaves of *S. rebaudiana*, t, various standards and stevia extract samples were submitted to Harvard University (FAS Small Molecule Mass Spectrometry Facility) for LC-MS analysis using the Synergy Hydro-RP column. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. The flow rate was 0.5 ml/minute. Mobile phase B was started at 20%B and maintained for 2 minutes. Then a linear gradient to 45% B over 15 minutes was run. Then %B was increased to 90% over 0.5 minutes and maintained for the following 8 minutes. The starting conditions were re-established over 0.5 minute and maintained for another 4.5 minutes prior to the next injection for re-equilibration of the column. Mass spectrometry analysis of the samples was done on the Bruker Impact II with an optimized method in positive ion mode. Funnel 1 RF was kept at 400Vp-p, Funnel 2 RF at 400 Vp-p, Transfer time of 120

μs and pre-pulse storage of 15 μs. A full calibration of the *m/z* scale was done with sodium formate clusters at the end of each run. All eluent was diverted to waste for the first 2 minutes using a secondary/optional 6-port valve to keep the source clean.

3. Results and Discussion

The molecular formula of compound **1** has been deduced as $C_{50}H_{80}O_{28}$ on the basis of its positive high resolution mass spectrum (HRMS) which showed adduct ions corresponding to $[M + NH_4]^+$ and $[M+ Na]^+$ at *m/z* 1146.5169 and 1151.4721 respectively (Figure 2); this composition was supported by the ^{13}C NMR spectral data. The ^1H NMR spectral data of **1** showed the presence of two methyl singlets at δ 1.10 and 1.44, two olefinic protons as singlets at δ 5.09 and 5.72 of an exocyclic double bond, nine sp3 methylene and two sp3 methine protons between δ 0.74 - 2.80, characteristic for the *ent*-kaurane diterpenoids isolated earlier from the genus *Stevia* [1]-[8]. The basic skeleton of *ent*-kaurane diterpenoids was supported by the TOCSY studies which showed key correlations: H-1/H-2; H-2/H-3; H-5/H-6; H-6/H-7; H-9/H-11; H-11/H-12. The ^1H NMR spectrum of **1** also showed the presence of anomeric protons resonating at δ 5.04, 5.10, 5.21, 5.48, and 6.30; suggesting five sugar units in its structure. Acid hydrolysis of **1** with 5% H_2SO_4 afforded D-glucose which was identified by direct comparison with authentic sample by TLC [14] [15] [16]. Enzymatic hydrolysis of compound 1 furnished an aglycone which was identified as steviol by comparison of ^1H-NMR and co-TLC with standard compound. The large coupling constants observed for the five anomeric protons of the glucose moieties at δ 5.04 (d, J = 7.5 Hz), 5.10 (d, J = 7.4 Hz), 5.21 (d, J = 7.9 Hz), 5.48 (d, J = 7.9 Hz), and 6.30 (d, J = 7.9 Hz), suggested their β-orientation as reported for steviol glycosides [1]-[6] [17] [18] [19] [20] [21]. The ^1H and ^{13}C NMR values for compound **1** were assigned on the basis of TOCSY, HMQC and HMBC data and are given in Table 1.

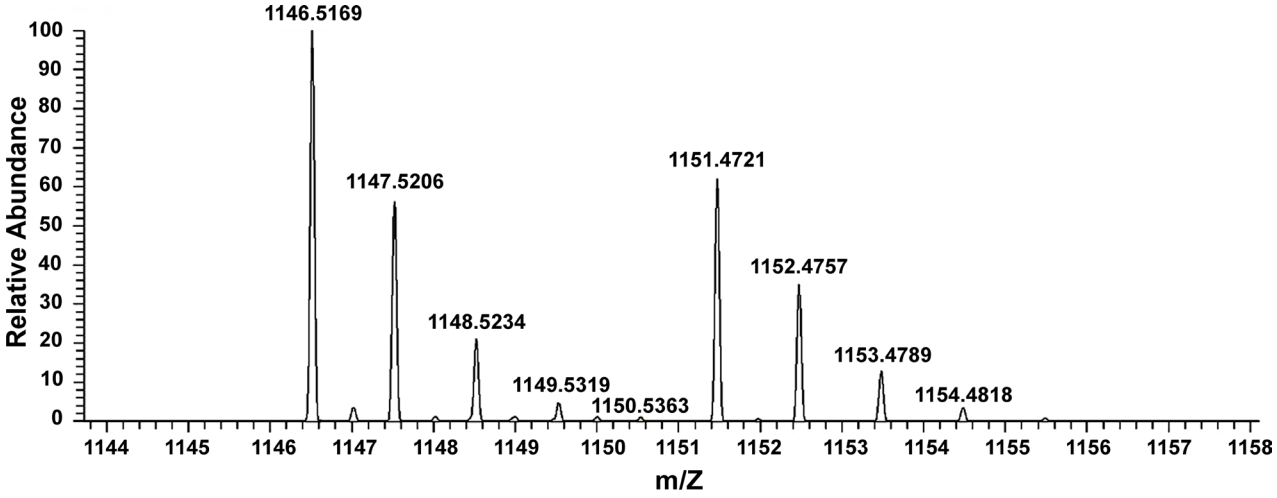

Figure 2. High Resolution Mass Spectral (HRMS) data of Reb D3 (**1**) indicating $[M + NH_4]^+$ and $[M + Na]^+$ adducts at *m/z* 1146.5169 and 1151.4721 respectively.

Table 1. ^{1}H and ^{13}C NMR spectral data (chemical shifts and coupling constants) for Reb D3 (1) and rebaudioside E (2)[a-c].

Position	Reb D3 (1)		Rebaudioside E (2)	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	0.74 t (12.8), 1.67 m	41.2	0.73 t (13.2), 1.68 m	41.0
2	1.48 m, 2.12 m	20.6	1.46 m, 2.13 m	20.6
3	1.13 m, 2.80 d (12.8)	38.4	1.12 m, 2.78 d (12.8)	38.2
4	---	44.9	---	44.8
5	0.98 d (11.8)	58.1	0.97 d (11.8)	57.9
6	1.87 m, 2.10 m	22.6	1.85 m, 2.09 m	22.6
7	1.28 m, 1.64 m	42.2	1.27 m, 1.63 m	42.1
8	---	43.3	---	43.0
9	0.88 d (7.5)	54.5	0.88 br s	54.5
10	---	40.3	---	40.2
11	1.66 m	21.2	1.65 m	21.1
12	1.91 m, 2.22 m	38.2	1.96 m, 2.16 m	37.8
13	---	86.8	---	86.6
14	1.69 d (11.4), 2.49 d (11.0)	44.9	1.74 d (11.4), 2.54 d (11.0)	44.8
15	2.04 m, 2.16 m	48.6	2.04 m, 2.12 m	48.5
16	---	155.0	---	154.9
17	5.09 s, 5.72 s	105.4	5.09 s, 5.76 s	105.4
18	1.44 s	29.9	1.43 s	29.8
19	---	176.3	---	176.2
20	1.10 s	17.3	1.10 s	17.2
1’	6.30 d (7.9)	93.9	6.30 d (7.9)	93.9
2’	4.38 m	81.5	4.38 m	81.7
3’	4.27 m	78.5	4.26 m	78.4
4’	4.24 m	72.0	4.22 m	72.1
5’	3.94 m	79.6	3.92 m	79.5
6’	4.33 m, 4.43 m	62.7	4.33 m, 4.43 m	62.6
1”	5.10 d (7.4)	98.3	5.16 d (7.5)	98.4
2”	4.18 m	84.8	4.17 m	84.9
3”	4.29 m	78.6	4.32 m	78.5
4”	4.20 m	71.3	4.22 m	71.8
5”	3.78 m	78.5	3.72 m	78.2
6”	4.32 m, 4.57 m	69.8	4.26 m, 4.35 m	62.9
1”’	5.21 d (7.9)	107.2	5.32 d (7.5)	107.2
2”’	4.14 t (8.4)	77.6	4.15 t (8.4)	77.7
3”’	4.25 m	78.7	4.26 m	78.6

Continued

4'''	4.34 m	72.4	4.36 m	72.3
5'''	3.94 m	79.1	3.96 m	79.0
6'''	4.43 m, 4.53 m	63.3	4.46 m, 4.56 m	63.2
1''''	5.48 d (7.9)	106.2	5.48 d (7.9)	106.2
2''''	4.04 t (7.9)	76.8	4.06 t (7.9)	76.8
3''''	4.22 m	78.8	4.25 m	78.7
4''''	4.32 m	71.2	4.31 m	71.2
5''''	3.99 m	79.1	4.02 m	79.1
6''''	4.38 m, 4.55 m	63.5	4.42 m, 4.54 m	63.4
1'''''	5.04 d (7.5)	105.9		
2'''''	4.02 m	77.0		
3'''''	4.21 m	78.6		
4'''''	4.25 m	72.2		
5'''''	3.96 m	79.1		
6'''''	4.34 m, 4.48 m	63.3		

[a]assignments made on the basis of TOCSY, HMQC and HMBC correlations; [b]Chemical shift values are in δ (ppm); [c]Coupling constants are in Hz.

Based on the results from NMR spectral data and hydrolysis experiments of **1**, it was concluded that there are five β-D-glucosyl units in its structure connected to the aglycone steviol. A close comparison of the ^{1}H and ^{13}C NMR values of **1** with rebaudioside E (**2**) (Table 1) as well as reported literature data [14] suggested the presence of a steviol aglycone moiety with a 2-*O*-β-D-glucobiosyl unit at C-13 in the form of an ether linkage and another 2-*O*-β-D-glucobiosyl unit at C-19 position in the form of an ester linkage, leaving the assignment of the additional β-D-glucosyl unit. Further, from the ^{13}C NMR spectral data of **1** which showed that one of the five oxymethine carbons of sugar moieties appeared downfiled at δ 69.8, suggested the placement of the additional β-D-glucosyl unit at this position. Identical proton and carbon spectral data for the two sugars I and IV in Reb D3 (**1**) and Rebaudiosde E (**2**) suggested the placement of the additional β-D-glucosyl unit at 6-position of either sugar II or sugar III. The downfield shift for both the ^{1}H and ^{13}C chemical shifts at 6-position of sugar II of the β-D-glucosyl moiety suggested the additional β-D-glucosyl unit has been attached at this position. The structure was further supported by the key TOCSY and HMBC correlations as shown in Figure 3. Based on the results of NMR and mass spectral data as well as hydrolysis studies, the structure of **1** produced by the enzymatic conversion of rebaudioside E was deduced as 13-[(2-*O*-β-D-glucopyranosyl-6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) ester.

The commercial stevia leaf extract prepared was analyzed by LC-MS to detect the existence of Reb D3. A minor compound at 7.3 min retention time (t_R) was identified as Reb D3 in the *Stevia* extract sample. The molecular formula of the

compound has been deduced as $C_{50}H_{80}O_{28}$ on the basis of its positive high resolution mass spectrum (HRMS) which showed adduct ions corresponding to $[M + NH_4]^+$ at m/z 1146.5174±0.005; identical to the known steviol glycoside isomer of rebaudioside D3, whereas rebaudioside D has a t_R of 9.5 min (Figure 4). These results provide the evidence to support the natural existence of the previously unidentified glycoside rebaudioside D3 in the commercial extracts of *S. rebaudiana*.

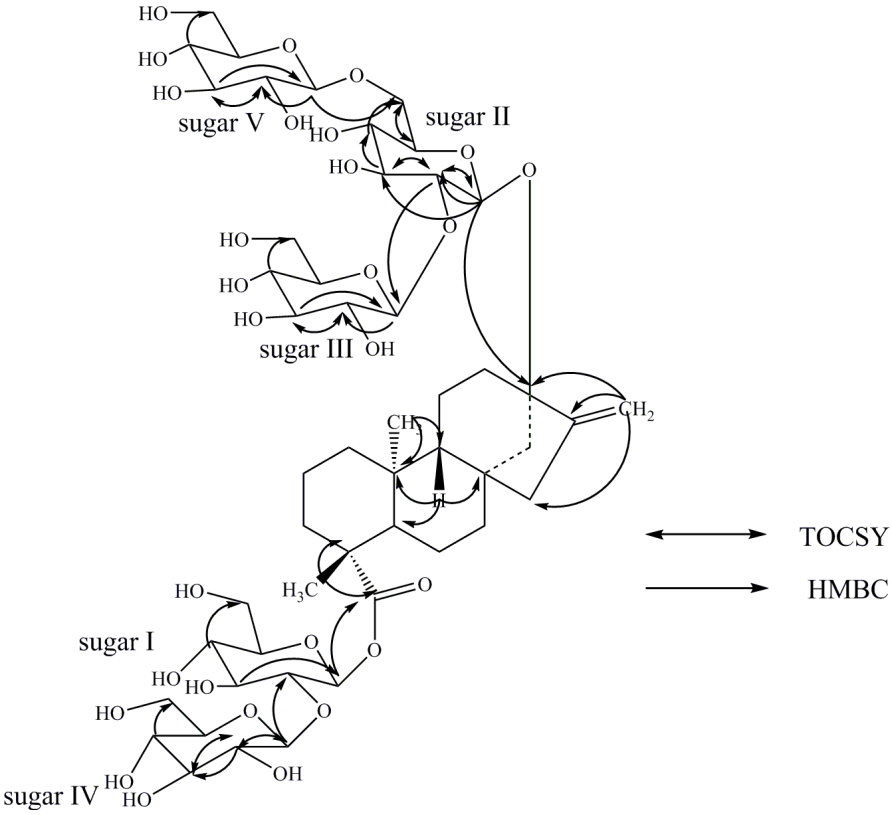

Figure 3. Key TOCSY and HMBC correlations of Reb D3 (**1**).

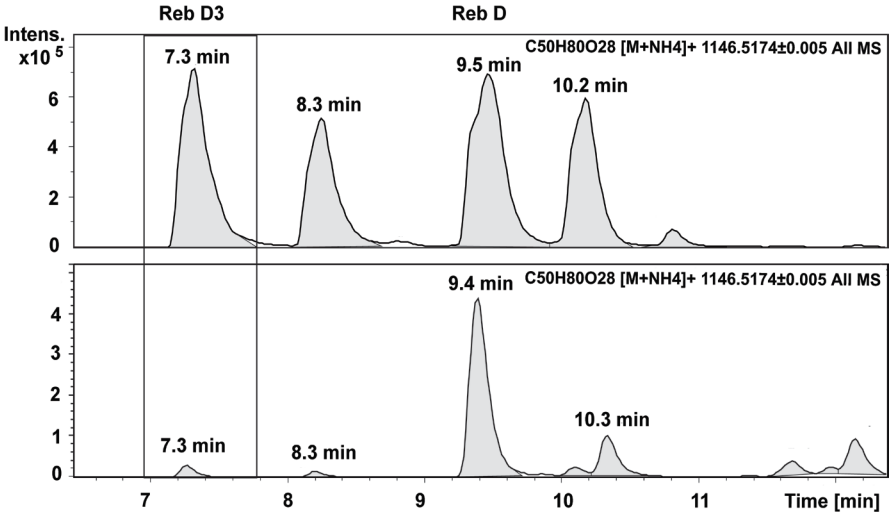

Figure 4. LC-MS analysis of the commercial extract of *Stevia rebaudiana* that identified the existence of Reb D3. Top panel corresponds to a mixture of steviol glycoside standards and bottom panel corresponds to an extract of *Stevia rebaudiana* leaves.

4. Conclusion

A new compound, named Reb D3 (**1**) was produced by the bioconversion of rebaudioside E using enzymatic methodology. The structure of **1** was confirmed as 13-[(2-*O*-*β*-D-glucopyranosyl-6-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) oxy] *ent*-kaur-16-en-19-oic acid-(2-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl) ester on the basis of extensive 1D (^{1}H and ^{13}C), and 2D NMR (TOCSY, HMQC, and HMBC) as well as high resolution mass spectral data and hydrolysis studies. The existence of **1** in the extract of *S. rebaudiana* leaves is confirmed by LC-MS of the crude stevia leaf extract, indicating that it is a natural steviol glycoside.

Acknowledgements

Thanks to Department of Chemistry, Emory University, Atlanta, GA and FAS Small Molecule Mass Spectrometry Facility, Harvard University, Cambridge, MA for providing NMR and LC-MS analysis respectively. This project is supported by Jiangsu International Collaboration Program BZ2014014, Wuxi City Grant CBE01G1407 and National 863-2013AA102801 to O.Y.

References

[1] Starratt, A.N., Kirby, C.W., Pocs, R. and Brandle, J.E. (2002) Rebaudioside F, a Diterepene Glycoside from *Stevia rebaudiana*. *Photochemistry*, **59**, 367-370. https://doi.org/10.1016/S0031-9422(01)00416-2

[2] Prakash, I. and Chaturvedula, V.S.P. (2013) Additional Minor Diterpene Glycosides from *Stevia rebaudiana* Bertoni. *Molecules*, **18**, 13510-13519. https://doi.org/10.3390/molecules181113510

[3] Prakash, I., Chaturvedula, V.S.P. and Markosyan, A. (2013) Isolation, Characterization and Sensory Evaluation of a Hexa *β*-D-Glucopyranosyl Diterpene from *Stevia rebaudiana*. *Natural Product Communications*, **8**, 1523-1526.

[4] Chaturvedula, V.S.P. and Prakash, I. (2011) A New Diterpenoid Glycoside from *Stevia rebaudiana*. *Molecules*, **16**, 2937-2943. https://doi.org/10.3390/molecules16042937

[5] Chaturvedula, V.S.P. and Prakash, I. (2011) Structures of the Novel Diterpene Glycosides from *Stevia rebaudiana*. *Carbohydrate Research*, **346**, 1057-1060. https://doi.org/10.1016/j.carres.2011.03.025

[6] Chaturvedula, V.S.P., Rhea, J., Milanowski, D., Mocek, U. and Prakash, I. (2011) Two Minor Diterpene Glycosides from the Leaves of *Stevia rebaudiana*. *Natural Product Communications*, **6**, 175-178.

[7] Madan, S., Ahmad, S., Singh, G.N., Kohli, K., Kumar, Y., Singh, R. and Garg, M. (2010) *Stevia rebaudiana* (Bert.) Bertoni—A Review. *Indian Journal of Natural Products and Resources*, **1**, 267-286.

[8] Kinghorn, A.D., Soejarto, D.D., Nanayakkara, N.P.D., Compadre, C.M., Makapugay, H.C., Hovanec-Brown, J.M., Medon, P.J. and Kamath, S.K. (1984) A Phytochemical Screening Procedure for Sweet Ent-Kaurene Glycosides in the Genus *Stevia*. *Journal of Natural Products*, **47**, 439-444. https://doi.org/10.1021/np50033a007

[9] Ohta, M., Sasa, S., Inoue, A., Tamai, T., Fujita, I., Morita, K. and Matsuura, F. (2010) Characterization of Novel Steviol Glycosides from Leaves of *Stevia rebaudiana* Morita. *Journal of Applied Glycosciences*, **57**, 199-209. https://doi.org/10.5458/jag.57.199

[10] Ibrahim, M.A., Rodenburg, D.L., Alves, K., Perera, W.H., Fronczek, F.R., Bowling, J. and McChesney, J.D. (2016) Rebaudiosides R and S, Minor Diterpene Glycosides from the Leaves of *Stevia rebaudiana*. *Journal of Natural Products*, **79**, 1468-1472. https://doi.org/10.1021/acs.jnatprod.6b00048

[11] Lairson, L.L., Henrissat, B., Davies, G.J. and Withers, S.G. (2008) Glycosyltransferases: Structure, Functions, and Mechanisms. *Annual Review of Biochemistry*, **77**, 521-555. https://doi.org/10.1146/annurev.biochem.76.061005.092322

[12] Yonekura-Sakakibara, K. and Hanada, K. (2011) An Evolutionary View of Functional Diversity in Family 1 Glycosyltransferases. *The Plant Journal*, **66**, 182-193. https://doi.org/10.1111/j.1365-313X.2011.04493.x

[13] Richman, A., Swanson, A., Humphrey, T., Chapman, R., McGarvey, B., Pocs, R. and Brandle, J. (2005) Functional Genomics Uncovers Three Glucosyltransferases Involved in the Synthesis of the Major Sweet Glucosides of *Stevia rebaudiana*. *The Plant Journal*, **41**, 56-67. https://doi.org/10.1111/j.1365-313X.2004.02275.x

[14] Bedir, E., Toyang, N.J., Khan, I.A., Walker, L.A. and Clark, A.M. (2001) A New Dammarane Type Triterpene Glycoside from *Polyscias fulva*. *Journal of Natural Products*, **64**, 95-97. https://doi.org/10.1021/np0003589

[15] Chaturvedula, V.S.P., Schilling, J.K., Miller, J.S., Andriantsiferana, R., Rasamison, V.E. and Kingston, D.G.I. (2003) New Cytotoxic Oleanane Saponins from the Infructescences of *Polyscias amplifolia* from the Madagascar Rainforest. *Planta Medica*, **69**, 440-444. https://doi.org/10.1055/s-2003-39711

[16] Huan, V.D., Yamamura, S., Ohtani, K., Kasai, R., Yamasaki, K., Nham, N.T. and Chau, H.M. (1998) Oleanane Saponins from *Polyscias fructicosa*. *Phytochemistry*, **47**, 451-457. https://doi.org/10.1016/S0031-9422(97)00618-3

[17] Chaturvedula, V.S.P. and Prakash, I. (2013) Structural Characterization and Hydrolysis Studies of Rebaudioside E, a Minor Sweet Component of *Stevia rebaudiana*. *European Chemical Bulletin*, **2**, 298-302.

[18] Chaturvedula, V.S.P., Chen, S., Yu, O. and Mao, G. (2013) NMR Spectral Analysis and Hydrolysis Studies of Rebaudioside N, a Minor Steviol Glycoside of *Stevia rebaudiana* Bertoni. *Food and Nutrition Sciences*, **4**, 1004-1008. https://doi.org/10.4236/fns.2013.410130

[19] Chaturvedula, V.S.P., Chen, S., Yu, O. and Mao, G. (2013) Isolation, NMR Spectral Analysis and Hydrolysis Studies of a Hepta Pyranosyl Diterpene Glycoside from *Stevia rebaudiana* Bertoni. *Biomolecules*, **3**, 733-740. https://doi.org/10.3390/biom3040733

[20] Chaturvedula, V.S.P., Yu, O. and Mao, G. (2013) Structural Characterization of the Hydrolysis Products of Rebaudioside M, a Minor Steviol Glycoside of *Stevia rebaudiana* Bertoni. *Journal of Chemical and Pharmaceutical Research*, **5**, 606-611.

[21] Chaturvedula, V.S.P., Yu, O. and Mao, G. (2013) NMR Spectral Analysis of Rebaudioside A, a Major Sweet Diterpene Glycoside of *Stevia rebaudiana* Bertoni at Various Temperatures. *International Journal of Pharmaceutical Science Invention*, **2**, 36-40.